PATENT
Docket No. 204372000320

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8-27-97 Alexandra H Parsons
Date Alexandra H. Parsons

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

DECLARATION OF GARY R. MATYAS, PH.D.
PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Gary R. Matyas, declare as follows:

1. I am engaged in medical research at the Walter Reed Army Institute of Research (WAIR) and have been active in studying cellular immune responses for 2 years. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

2. Under my supervision, studies were performed using Balb/C mice. In order to generate cytotoxic T lymphocytes (CTL) in response to recombinant human prostate specific antigen (rhPSA) we immunized two groups of six mice per group with OncoVaxP™ alone or with OncoVaxP™ mixed with aluminum hydroxide (alum, Alhydrogel, 200 μg Al^{+++}).

dc-85046

OncoVaxP™ is a liposomal formulation of rhPSA with lipid A, and the PSA dose in this protocol was 5 μg rhPSA and 20 μg lipid A. The mice were immunized at week 0 and week 4 at these dosages and euthanized at 8 weeks. The spleens were removed and spleen cells harvested, pooled and incubated for 5 days either with medium alone or in medium containing 2 μg/ml rhPSA.

3. Target cells were P815 mouse mastocytoma cells infected either with wild-type vaccinia virus or with vaccinia virus transfected with the PSA gene and the *E. coli* lacZ gene (PSAVac, Therion Biologics Corp.) at a multiplicity of infection of 10. Alternatively, the P815 cells were incubated with 10 μg/ml of the peptide CYASGWGSI which represents a potential CTL epitope at positions 153-161 of PSA.

4. After incubation for 16 hours with vaccinia viruses or peptide, the target cells were labeled with Cr^{51} (0.1 μCi/10^6 cells) for 1 hour).

5. The effector cells harvested from the moue spleens were plated in 96-well u-bottom plates and the Cr^{51} labeled target 815 cells were added at various effector:target ratios. The plates were centrifuged at 50xg for 5 minutes and then incubated at 37°C for 5 hours. The radioactivity present in the supernatant (indicative of cytotoxic activity of the effector cells) was harvested using Skatron wicks and quantified using a gamma counter.

6. The attached Exhibit B shows the results. As shown, spleen cells from mice injected with either OncoVaxP™ or OncoVaxP™ with alum were able to lyse target cells that had been transfected with the vaccinia virus (Panels A and B). OncoVaxP™ injected mice in the absence of alum (panel E) were effective in lysing the targets labeled with peptide, although when alum was included in the formulation, the resulting spleen cells were less able to do so (panel F). P815 target cells unlabeled with either peptide or vaccinia-produced PSA were not lysed (panels C, D, G, and H).

7. These results demonstrate that immunization of mice with OncoVaxP™ induces lymphocytes which kill tumor cells presenting PSA antigens, indicating an antitumor response.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Washington, D.C. on 26 August 1997, by

Gary R. Matyas

August , 1997

CURRICULUM VITAE

Gary R. Matyas

Office:
Department of Membrane Biochemistry
Walter Reed Army Institute of Research
Washington DC 20307-5100
(202) 782-0875

Home:
18415 Snowberry Way
Olney, MD 20832
(301) 570-0610

CAREER AIMS: Research/management of membrane biochemistry laboratory with emphasis on liposome based vaccines, lipid mediators, and lipid changes during cell growth.

PERSONAL: Born April 30, 1956 in Berwick, PA; married, one child

EDUCATION:

Graduate
Purdue University, West Lafayette, IN 47907
Ph.D. degree; May 1985; Department of Biological Sciences
Major Professor: D. James Morré

Undergraduate
Pennsylvania State University, University Park, PA 16802
B.S. degree; May 1978; Major - Biophysics

EXPERIENCE:

Research

Research Chemist
Department of Membrane Biochemistry
Division of Biochemistry
Walter Reed Army Institute of Research
Washington DC 20307
September 1988 to present
Supervisor: Dr. Carl R. Alving

Projects:

I. Development of active site monoclonal antibodies to cobra venom phospholipase A_2: Inhibitors of enzyme activity

WRAIR Projects (cont.):

II Mechanism of concanavalin A induced killing of mice and cultured cells

II Incorporation of bioactive lipids into liposomes: Effect on immune response to liposome encapsulated antigens

IV Development of monoclonal antibodies to sphingosine and sphingolipids

V Development of a liposomal vaccine against ricin intoxication

VI In collaboration with Jenner Technologies, development, manufacture and testing of liposomal based cancer vaccine in human clinical trials

VII Development of a liposomal vaccine protects against Ebola virus infection through the induction of cytotoxic lymphocytes.

Additional Duties:

I. Division of Biochemistry Safety Officer, Division representative to WRAIR safety council; Responsibilities include: Monthly safety inspections of division laboratories; Conducting training on safety concerns; Maintaining division training records.

II. Terminal Security Officer for the Department of Membrane Biochemistry; Duties include: Maintaining security on computer equipment in the department; Procuring of IBM compatible equipment and software.

Staff Fellow
Membrane Biochemistry Section
Laboratory of Molecular and Cellular Neurobiology/Developmental and Metabolic Neurology Branch
National Institute of Neurological, Communicative Disorders and Stroke
National Institutes of Health
Bethesda, MD 20892
April 1985 to September 1988
Supervisor: Dr. Peter Fishman

Projects:

I. Glycolipid alterations induced by transfection of NIH/3T3 cells with oncogenes.

II. Alterations of glycolipids during cell growth.

NIH projects (cont.)

II Production of glycolipid crosslinking reagents for the study of the involvement of glycolipids in cell adhesion and cell growth.

IV. The role of *ras* oncogenes in phospholipase C mediated phosphoinositide hydrolysis.

Graduate Assistant
Department of Biological Sciences
Purdue University
West Lafayette, IN 47907
August 1978 to April 1985
Supervisor: Dr. D. James Morré

Projects:

I. Interaction of fibronectin with gangliosides.

II Subcellular distribution and biosynthesis of gangliosides in rat liver.

III. Loss of fibronectin and complex gangliosides in metastatic rat tumors.

IV. Elevated levels of serum gangliosides as a means of early detection of cancer.

V. Cytochemical localization of glycosyltransferases.

Research Assistant
Department of Biochemistry and Biophysics
Pennsylvania State University
University Park, PA 16802
September 1977 to May 1988
Supervisor: Dr. Wallace Snipes

Project:

I. Inactivation of lipid-containing viruses through physical perturbation of membranes.

Teaching

Instructor
Introductory Microbiology Laboratory
Department of Biological Sciences
Purdue University
West Lafayette, IN 47907
January 1981 to May 1981
Supervisor: Dr. David Filmer

Teaching Assistant
Introductory Microbiology Laboratory
Department of Biological Sciences
Purdue University
West Lafayette, IN 47907
August 1979 to May 1980, August 1978 to May 1979
Supervisors: Dr. Allen Konopka and Dr. David Filmer

AWARDS:

Special Purdue Fellowship
Purdue University
West Lafayette, IN 47907
August 1980 to December 1980; $2,000

Marion County Cancer Society (Little Red Door) Fellowship
1801 North Meridian Street, Indianapolis, IN 46202
"A New Serodiagnostic Parameter for Early Detection of Cancer That May Distinguish Localized and Disseminated Disease"
January 1982 to December 1982; $12,498

Milheim Foundation for Cancer Research
Colorado National Bank
Seventeenth Street at Champa
Denver, CO 80202
"The Biochemical Basis for Metastasis"
July 1982 to June 1983; $11,421

David Ross Fellowship
Purdue University
West Lafayette, IN 47907
"Protein Kinase Modulations as Early Events of Tumorigenic Progression"
August 1983 to July 1985; $13,200

MEMBERSHIPS:

American Association for the Advancement of Science
1978 to present

American Society for Biochemistry and Molecular Biology
1987 to present

American Society of Microbiology
1996 to present

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Gary R. Matyas

PUBLICATIONS:

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2. Matyas, G. R. and Morre, D. J.: Coupling of Uridine-5'-Diphosphate (UDP) Formation and Nicotinamide Adenine Dinucleotide (NADH) Reduction for Cytochemical Localization of Glycosyltransferases. Journal of Histochemistry and Cytochemistry (1983) 31, 1175-1182.

3. Matyas, G. R. and Morre, D. J.: Isolation of Purified Membranes and Membranous Cell Components for Receptor Studies. Investigations of Membrane-Located Receptors (Methodological Surveys (B): Biochemistry Vol. 12, Eds. Reid, E., Cook, G. M. W. and Morré, D. J., Plenum Publishing Corp., New York, NY (1984) pp. 111-118.

4. Morre, D. J., Creek, K. E., Matyas, G. R., Minnifield, N., Sun, I., Baudoin, P., Morré, D. M. and Crane, F. L.: Free-flow Electrophoresis for Subfractionation of Rat Liver Golgi Apparatus. Biotechniques (1984) 2, 224-233.

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6. Matyas, G. R., Walter-Doelling, V. P., Ferroli, C., Pennington, K. R., Pikaard, D. and Morre, D. J.: Glycolipid Antigens: Potential in Cancer Detection. Investigation and Exploitation of Antibody Combining Sites (Methodological Surveys (B): Biochemistry Vol. 14, Eds. Reid, E., Cook, G. M. W. and Morre, D. J., Plenum Publishing Corp., New York, NY (1986) pp. 323-332.

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13. Matyas, G. R., and Fishman, P. H.: Lipid Signalling Pathways in Normal and *Ras*-Transfected NIH/3T3 Cells. Cellular Signalling (1989) 1, 395-404.

14. Buckley, N. E., Matyas, G. R. and Spiegel, S.: The Bimodual Growth Response of Swiss 3T3 Cells to the B Subunit of Cholera Toxin Is Independent of the Density of Its Receptor, Ganglioside, GM1. Experimental Cell Research (1990) 189, 13-21.

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18. Gregory M. Glenn, Mangala Rao, Roberta L. Richards, Gary R. Matyas, and Carl A. Alving. Murine IgG Subclass Antibodies to Antigens Incorporated in Liposomes Containing Lipid A.. Immunological Letters (1995) 47, 73-78.

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20. Gary R. Matyas and Carl R. Alving. Protective Prophylactic Immunity Against Intranasal Ricin Challenge Induced by Liposomal Ricin A Subunit. Vaccine Research (1996) 5:163-172.

MANUSCRIPTS SUBMITTED:

1. Gregory M. Glenn, Mangala Rao, Gary R. Matyas, Patricia Walker and Carl R. Alving. Transcutaneous Immunization Using Bacterial ADP-Ribosylating Exotoxin. Science (submitted).

2. Mangala Rao, Gary R. Matyas, Franziska Grieder, Kevin Anderson, Peter B. Jahrling, and Carl R. Alving. Cytotoxic T Lymphocytes to Ebola Zaire Virus Are Induced in Mice by Immunization with Liposomes Containing Lipid A. European Journal of Immunology (submitted).

ABSTRACTS:

1. Matyas, G. R. and Werderitsh, D. A.: Comparison and Ganglioside Composition of Metastatic and Non-metastatic Transplantable Tumors from the Jaw Region of the Rat. Federation Proceedings (1981) 40, 1544.

2. Morré, D. J., Hsu, H., Matyas, G. R., Kloppel, T., Classen, P. and Jacobson, W.: Lipid-associated Sialic Acid (LASA) Elevated in Sera of Carcinoma Patients. Proceedings of the American Association of Cancer Research (1981) 22, 22.

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22. Mayorga, M. A., Matyas, G. R., Wihelmsen, C. L., Banks, R. E. and Alving, C. R.: Acute Respiratory Injury Induced By Endotracheally Instilled Snake Venom Phospholipase A_2 (PLA_2). The FASEB Journal (1991) 5, 1268.

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26. Matyas, G. R. and Alving, C. R.: Effect of Route of Administration on Generation of Different Isotype Antibodies to Liposome-Encapsulated Ricin Subunits. The FASEB Journal (1994) 8, A1464.

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Gary R. Matyas

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29. Matyas, G. R. and Alving, C. R.: Production of Serum IgA and High Titer IgG Antibodies by Intranasal Immunization with Liposome-Encapsulated Ricin Subunits. The FASEB Journal (1995) 9, A216.

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INDUCTION OF CYTOTOXIC T-LYMPHOCYTES TO TUMOR CELLS PRESENTING PROSTATE SPECIFIC ANTIGENS

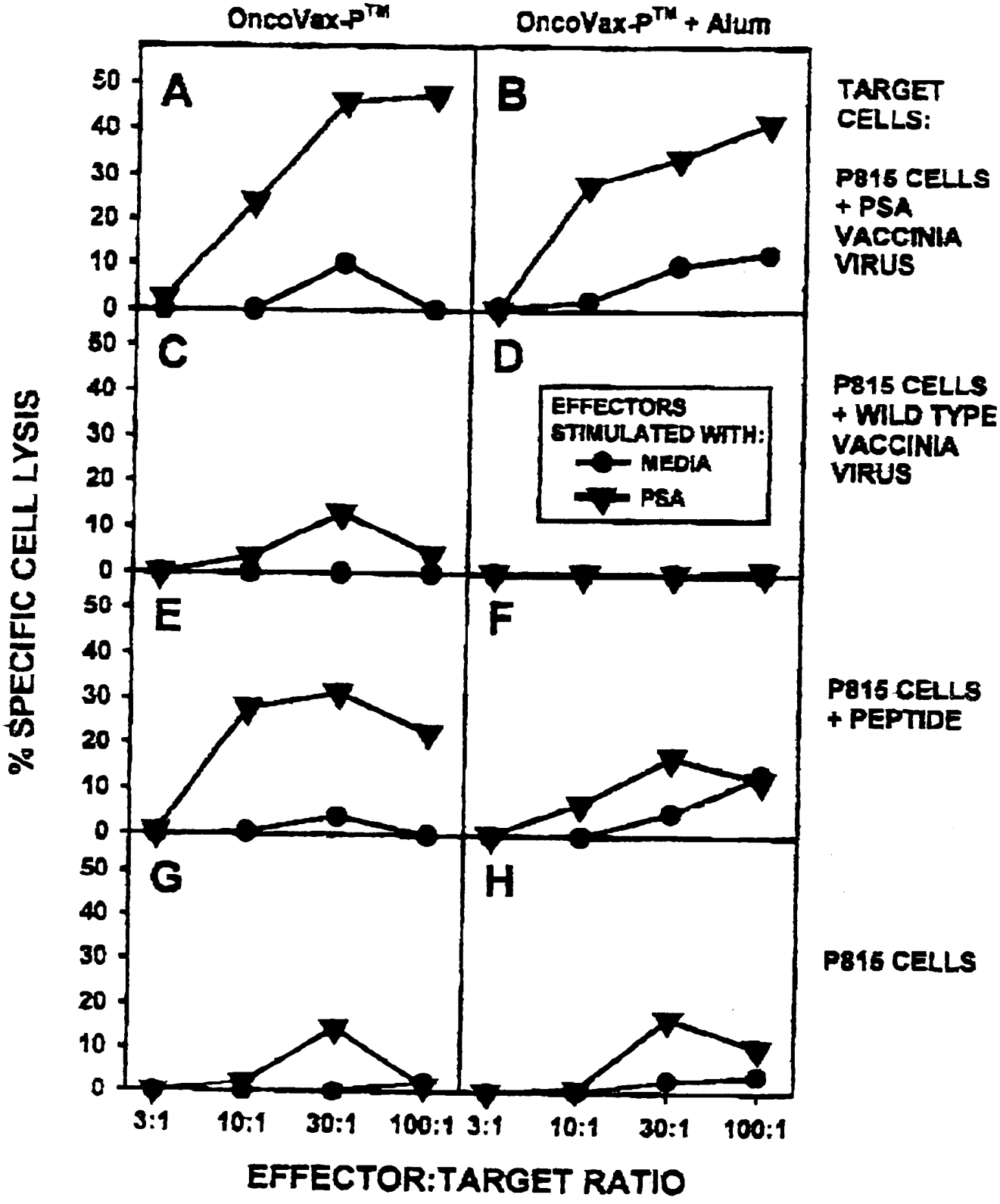

Exhibit B

Matyas

Induction of Antitumor Response in Mice by Prostate Cancer Vaccine

Gary Matyas. PhD, Mangala Rao, PhD, Jean Muderhwa, PhD. and Carl Alving,MD, Walter Reed Army Institute of Research, Washington, DC

We report that immunization of mice with OncoVax-P™ prostate cancer vaccine induces cytotoxic T-lymphocytes which have the capability of killing tumor cells presenting PSA antigens.

Two groups of Balb/c mice (6 mice/group) were immunized with OncoVax-P™. One group was immunized with OncoVax-P™ alone and the other group received OncoVax-P™ mixed with aluminum hydroxide (alum, Alhydrogel) (200 μg Al^{+++}). OncoVax-P™ is a liposomal formulation of prostate specific antigen (PSA) with lipid A, as an adjuvant. The PSA dose was 5 μg PSA and 20 μg of lipid A. The mice were immunized at week 0 and 4 weeks after the boost. Three animals per group were euthanized 4 weeks after the boost and the spleens were removed for CTL assay. The cells from each group of spleens were pooled and incubated 5 days with media alone or 2 μg/ml human PSA. Target P815 cells (mouse mastocytoma) were infected with either wild type vaccinia virus (WTvac) or with vaccinia virus transfected with PSA gene and the *E. coli* lac Z gene (PSAvac, Therion Biologics Corporation) at an multiplicity of infection of 10. P815 cells were incubated with 10 μg/ml of peptide, CYASGWGSI, which represents a potential murine CTL epitope for PSA. It was identified using the University of Wisconsin Genetics Computer Group sequence analysis finding pasterns computer program. This program was used to compare the amino acid sequence of PSA with the known murine CTL anchor sequences. CYASGWGSI is located at amino acid positions 128-137 of PSA. The P815 targets were incubated for 16 hours with vaccinia viruses or peptide. The targets cells were then labeled with Cr^{51} (0.1 μCi/10^6 cells) for one hour. Effector cells were harvested and plated in 96 well U bottom plates. Cr^{51} labeled targets were added at various effector:target ratios. Following centrifugation at approximately 50 X g for 5 min, the plates were incubated at 37°C for 5 hr. The radioactivity present in the supernatant was harvested using Skatron wicks and the radioactivity was quantified using a gamma counter.

As shown in the attached figure, murine CTL which specifically lysed PSAvac infected targets (A & B) and targets incubated with peptide (E) were obtained. Approximately, 47% cell lysis was obtained using PSAvac targets. WTvac targets were not lysed (C & D), indicating the CTLs were specific for PSA epitopes. Addition of alum to the vaccine had no effect on the generation of CTL which lysed PSAvac targets (A & B) but it inhibited the induction of CTLs to tumor cells pulsed with peptide.

These results indicate that immunization of mice with the prostate cancer vaccine induces lymphocytes capable of specifically killing tumor cells presenting PSA antigens indicating an antitumor response.